

# Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua*, on cotton

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## Abstract

Nitrogen (N) is one of the most critical chemical elements for plant and animal growth, exerting a variety of bottom-up effects. Development and oviposition of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), were studied in relation to varying N fertilization levels (42, 112, 196, and 280 p.p.m.) in cotton [*Gossypium hirsutum* L. (Malvaceae)]. Low N fertilization of cotton plants led to reduced plant biomass and a lower percentage of N in leaf blades and in leaf petioles. Development of *S. exigua* larvae fed with plants with reduced N applications (42 and 112 p.p.m.) was prolonged relative to treatments receiving higher N fertilization. Almost all larvae reared on artificial diets underwent only five instars before pupation. However, most larvae reared on cotton plants, irrespective of N levels, experienced a supernumerary sixth larval instar. Furthermore, significantly more larvae reared on lower N cotton plants underwent supernumerary development compared to larvae reared on higher N cotton plants. Life-time feeding damage per larva ranged from 55 to 65 cm<sup>2</sup>, depending on the nutritional quality of the food plant, although the differences were not statistically significant. Larvae distinguished between cotton plants with various nutritional qualities and fed preferentially on higher N plants. Female moth oviposition choice was also affected by host plant nutritional quality: cotton plants with higher N levels were preferentially chosen by *S. exigua* females for oviposition. The mechanisms of these effects are unclear, but they can have important implications for population dynamics and pest status of beet armyworms in the field.

## Introduction

Nitrogen (N) is a critical element for plants and herbivores, profoundly influencing development and reproduction. Plants with added N typically grow more vigorously, to an upper limit. They also have greater N content, expressed as percentage N of dried mass or total protein, and biomass (Dudt & Shure, 1994; Wilkens et al., 1996; Darrow & Bowers, 1999; Glynn et al., 2003; Stiling & Moon, 2005). Correspondingly, insects that feed on diets or host plants high in N generally have greater growth rates, higher efficiency of conversion of ingested food, and shorter

developmental times (Mattson, 1980; Lindroth et al., 1995; Chen et al., 2004).

Many herbivorous insects can qualitatively distinguish among host plants, and feed and oviposit preferentially on high quality plants (White, 1984; Fox et al., 1990; Jauset et al., 1998; Chen et al., 2004; Prudic et al., 2005). For instance, females of the buckeye butterfly, *Junonia coenia* Hübner (Lepidoptera: Nymphalidae), selected fertilized over unfertilized host plants for oviposition (Prudic et al., 2005). Whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), females preferred to oviposit on tomato plants [*Lycopersicon esculentum* Mill. (Solanaceae)] with high N levels over those with low N (Jauset et al., 1998). Therefore, high N fertilization might contribute to higher pest populations in crops and may lead to yield loss due to preferential feeding. In cropping systems, high N inputs also increase production costs and environmental

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risks such as contamination of water resources due to fertilizer run-off (Kyllmar et al., 2006; Ojeda et al., 2006; Udawatta et al., 2006).

In contrast to the situation on plants with high N, phytophagous insects feeding on low N plants generally have prolonged developmental times, lower growth rates, and potentially increased windows of vulnerability to their natural antagonists. These changes may result in higher herbivore mortality (termed the 'slow-growth high-mortality' hypothesis; Feeny, 1976; Moran & Hamilton, 1980; Benrey & Denno, 1997, but see Clancy & Price, 1987; Williams, 1999). Therefore, reduced plant biomass due to low N might be compensated for by higher mortality of pests due to natural enemies.

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is a generalist herbivore with over 90 known host plant species, including a number of economically important crops, such as tomatoes, cotton, corn, soybeans, peanuts, and peppers (Pearson, 1982). Beet armyworm populations are often suppressed by a complex of parasitoids, predators, and pathogens (Ruberson et al., 1994a; Mohaghegh et al., 2001; Bianchi et al., 2002; Ehler, 2007). However, beet armyworm outbreaks can be triggered by applications of insecticides that deplete or remove the natural enemy complex (Eveleens et al., 1973; Ruberson et al., 1994a). Severe beet armyworm outbreaks in cotton have occurred in the USA intermittently from the late 1980s, but the factors involved in those outbreaks are poorly understood.

In this study, we investigated the impact of N fertilization on cotton plant growth and beet armyworm feeding, growth, and oviposition behavior. Specifically, we tested four hypotheses: (i) beet armyworm development will be prolonged due to lower host plant N fertilization, (ii) the life-time feeding damage of beet armyworm will be inversely related to host plant N fertilization rates, (iii) beet armyworm larvae will prefer to feed on host plant tissues receiving higher N fertilization, and (iv) beet armyworm females will oviposit preferentially on host plants with higher N fertilization.

## Materials and methods

Cotton plants, *Gossypium hirsutum* L. (Malvaceae) (cultivar FiberMax 989), were individually grown in 1-l pots using sphagnum peat moss (Premier Horticulture Inc., Quakertown, PA, USA) and landscape top soil (Hood Timber Co., Adel, GA, USA) with a ratio of 3:1 as a growing medium in the greenhouse. The photoperiod was L14:D10. The temperature was set at approximately 32 °C during the day and 28 °C during the night.

Four growing regimes (treatments) were utilized, all involving manipulations of N in the water solution

**Table 1** Macronutrient solution formulas (ml l<sup>-1</sup> water) for generating four N levels (modified Hoagland solution: Hoagland & Arnon, 1950)

Macronutrients	N treatments (p.p.m. N)			
	42	112	196	280
NH <sub>4</sub> NO <sub>3</sub> (1 M)	0.5	1	2	5
Ca(NO <sub>3</sub> ) <sub>2</sub> (1 M)	1	3	5	5
KH <sub>2</sub> PO <sub>4</sub> (1 M)	4	4	4	4
MgSO <sub>4</sub> (1 M)	2	2	2	2

comprising 42, 112, 196, and 280 p.p.m. N (see Table 1 for macronutrient formulas). The micronutrients contained in all N treatments were 1 ml each of MnCl<sub>2</sub> (0.004 M), CuSO<sub>4</sub> (0.0003 M), H<sub>3</sub>BO<sub>3</sub> (0.05 M), MoO<sub>3</sub> (0.0001 M), ZnSO<sub>4</sub> (0.0008 M), and Fe sequestrene (10% Fe<sup>3+</sup>) sodium ferric diethylenetriamine penta-acetate in 1 l of water. The four N treatments in p.p.m. are roughly equivalent to 19, 50, 87, and 125 kg ha<sup>-1</sup> N in the field, respectively. The nutrient solutions with varying N levels were generated by altering the volumes of the first two macronutrient chemicals in the solutions to maintain the ratios of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> forms of N within the desirable range (Jones, 1997).

Following seedling emergence, cotton plants were watered daily with 100 ml of 112 p.p.m. N nutrient solution for ca. 2 weeks, when the plants attained the two-true-leaf stage (the third leaf was still small), at which time plants were assigned to the different treatments and were fertilized with appropriate nutrient solutions. The experimental design was a randomized complete block. Plants were first arranged into blocks by matching leaf size and plant height. Within a block, plants were randomly assigned to each treatment. Plants were fertilized 5–6 times weekly. Leaching (watering without nutrients) followed every fourth nutrient solution application in order to reduce salt (salinity) build-up. About 2 weeks later and at the time of the experiments, cotton plants had 3–5 mature true leaves. All plants used in the study were prepared this way and were at this stage unless otherwise noted. Beet armyworm larvae and adults used in the experiments were laboratory reared on modified Pinto bean diet (Burton, 1969) at 25 ± 1 °C and a photoperiod of L14:D10, except otherwise noted.

## Nitrogen effect on cotton plant growth and nutritional quality

We first characterized the effects of the N treatments on the cotton plants. Nitrogen effects on plant height and above- and below-ground plant biomass were examined on 20 cotton plants (in four blocks). At the time of the experiment, plant height from the first lateral root to the top of the terminal was measured. Plant height at time of

first treatment fertilization was determined as a covariate. Above- and below-ground fresh plant material was then cleaned and dried in an oven at 65 °C for 2 days before weighing.

Plant tissue N analysis was conducted on 30 plants for each treatment. Leaf blades of the same leaf position for a group of six plants per treatment were pooled to assess total N. Likewise, leaf petioles of the same treatment within a group were pooled for total nitrate-N analysis. Leaf blade samples were separately prepared by leaf positions [true leaves 1 and 2 combined (L1/2), true leaf 4 (L4), and true leaf 5 (L5)], to understand the within-plant pattern of N, and analyzed for percentage of total N as a percentage of dry mass. Samples were immediately oven dried at 65 °C for 2 days before submission to the Soil, Plant, and Water Laboratory at the University of Georgia for N analysis. Total N plant tissue analysis uses a sulfuric ( $\text{H}_2\text{SO}_4$ )–salicylic ( $\text{C}_7\text{H}_6\text{O}_3$ ) acid mixture as a digestion reagent (Buresh et al., 1982). Petiole tissue nitrate-N analysis uses a  $\text{H}_2\text{O}_2$ – $\text{H}_2\text{SO}_4$  mixture for digestion of plant material in the absence of heavy metals that were previously used in the plant and soil analysis (McGill & Figureiredo, 1993).

*Hypothesis 1: beet armyworm development will be prolonged due to lower host plant N fertilization.* Two trials were set up to assay short-term larval development on cotton plants of different N levels. In the first trial, neonate larvae were bioassayed in Petri dishes kept in an environmental chamber at  $25 \pm 1$  °C and photoperiod of L14:D10. Development of individual larvae can vary significantly even on the same diet with all other environmental variables held identical (Y Chen, pers. obs.). In order to stabilize variance, groups of 10 larvae were kept in Petri dishes supplied with an excised cotton leaf that was changed daily. Leaves from different treatments used on the same day were from the same leaf node (true leaf 1, 2, 3, and 4 from the beginning to the end). Larvae were weighed after 1, 3, and 4 days in groups of 10, when larvae were 1, 3, and 4 days old, respectively.

To simulate *S. exigua* normal feeding behavior in the nature, a second trial was conducted in which larvae were allowed to move freely throughout the caged plant. In this trial, 10 3-day-old (reared on artificial diets for the first 3 days following eclosion) larvae were put on the second true leaf of cotton plants that were individually caged in a mesh bag in the greenhouse. We used 3-day-old larvae instead of neonate larvae to reduce loss from dislodgement and to facilitate manipulation. The greenhouse conditions were the same as described above. Larvae from each plant were removed after 4 days of feeding and weighed as a group. They were put back on the second true leaf after weighing. Larvae were collected and weighed again 3 days

later. Because most of the leaves of caged plants were consumed after 7 days of feeding, recovered larvae were subsequently kept on modified Pinto bean diet (Burton, 1969) to equalize rearing conditions, in groups of 4–5 larvae per diet cup until pupation. Adult emergence was recorded. In both trials, we used a randomized complete block design. Cotton plants were arranged into eight blocks before randomly assigning them to four treatments within each block.

To examine the impact of host plant N levels on life-time development of larvae, neonate larvae (less than 16 h old) were reared individually in Petri dishes (diameter = 50 mm; Becton Dickinson and Company, Franklin Lakes, NJ, USA) with excised leaf tissue from cotton plants of 42 and 196 p.p.m. N treatments in an environmental chamber set at  $25 \pm 1$  °C and with a photoperiod of L14:D10. The leaf tissue used was obtained from node 1, but leaf tissue from higher nodes was progressively used as leaves were depleted. Leaf tissue used for all treatments was from the same node on the same day to reduce variability. A small cotton ball soaked with water was placed inside the dish to maintain humidity and the turgidity of leaf tissue. Larvae were not observed feeding or resting on the cotton ball. Fresh leaf tissue was replaced once or twice daily. Larvae were checked daily for molting until pupation. Cast head capsules were measured with an ocular micrometer in a stereomicroscope (model Wild M3C; Leica Microsystems Ltd., Heerbrugg, Switzerland) under 40× magnification, to determine instar. The number of instars each larva went through before pupation was recorded. Each treatment was replicated five times with 10 individually reared larvae per replicate for a total of 50 larvae.

*Hypothesis 2: the life-time feeding damage of beet armyworm will be inversely related to host plant N fertilization rates.* The experiment was conducted in the greenhouse described above. Three 3-day-old larvae were caged on the first true leaf of each plant for 2–3 days. Three-day-old larvae were used, because they were easy to handle, could not easily escape the cages, and fed sufficiently to permit measurement. Cages were of a clamshell design made with plastic soft-drink lids in the center of which a disk was cut out and covered with fine mesh gauze (see Chen et al., 2006, for details). Larvae were progressively moved to the next upper leaf as they consumed the leaves on which they were caged. Larvae of all treatments were moved to new leaves of the same node on the same day to manage variability. The leaf area eaten was measured 2, 4, and 7 days after initial caging (see Chen et al., 2006), and cumulative damage to pupation was also measured, which corresponded to leaf tissue consumed within 5, 7, 10 days, and pre-imaginal life-time (feeding in the first 3 days following eclosion is

negligible). A randomized complete block design was used with 10 blocks (replicates; three larvae/block) and the four N treatments described above.

*Hypothesis 3: beet armyworm larvae will prefer to feed on host plant tissues receiving higher N fertilization.* Larval feeding preference was evaluated in cages made of two wooden rings (diameter = 30 cm). The rim of each ring was 2 cm wide and 1.8 cm high with two small notches cut into opposite sides of the ring to accommodate leaf petioles. One side of each ring was covered with fine mesh, and the two rings were clamped together to enclose the experimental leaves and larvae. The petioles of the third true leaf from plants of two different N treatments were clamped into the cages wrapped with cotton to prevent escape of the larvae. Eight 5-day-old larvae were placed in the middle of each arena and were recovered 24 h later. The number of larvae on each leaf was recorded at recovery.

Six combinations of two-choice tests among N treatments were conducted. These were 42 vs. 112 p.p.m., 42 vs. 196 p.p.m., 42 vs. 280 p.p.m., 112 vs. 196 p.p.m., 42 vs. 280 p.p.m., and 196 vs. 280 p.p.m. N. Each combination was replicated four times.

*Hypothesis 4: beet armyworm females will oviposit preferentially on host plants with higher N fertilization.* Pupae were obtained from the laboratory colony reared on artificial diet. Pupae were sexed and pupae of the same sex were kept in the same cup. After adult emergence, one male and one female were paired and kept in a 5-ml diet cup. Maximum egg production by female *S. exigua* occurs in the first 1–2 nights following initial oviposition (Fye & McAda, 1972), so females were used immediately following their initial oviposition in the cups. All females used were 3–5 days old.

The same test arena used to assess larval feeding preference was used in the tests. The petioles of the third true leaves from two different treatments were placed in the ring cages. Petioles were wrapped with cotton to avoid escape of the adult female. One gravid female *S. exigua* was released in the middle of each test arena and allowed to choose between cotton leaves from two N treatments. The test leaves were checked for egg masses 24 h later. The number of egg masses and the number of eggs on each leaf were recorded. The experiment was repeated eight times for each pairing. The N treatments tested in the experiment were 42, 112, and 196 p.p.m. N. The two-choice tests were 42 vs. 196 p.p.m. N and 112 vs. 196 p.p.m. N. All tests were done in the greenhouse described previously.

#### Experimental design and statistical analysis

Plant height, dry shoot and root weights, total plant mass, and nitrate-N were analyzed with one-way analysis of

variance (ANOVA) (SAS Institute, 1999). Percentage of total N of dried leaf blades was analyzed with a non-parametric Kruskal–Wallis test (SAS Institute, 1999). Larval weight, time to pupation, and time to adult emergence in relation to N levels were analyzed by one-way ANOVA. Percentage of *S. exigua* larvae pupated in the free-moving developmental trial was analyzed with Kruskal–Wallis test. Progressive larval weights were analyzed with repeated measures ANOVA, with weighing dates as the repeated measure. Data in supernumerary development experiment were analyzed with one-way ANOVA. Percentages of larvae undergoing 5 or 6 instars before pupation were transformed ( $\arcsin \sqrt{x}$ ) before analysis. In the larval preference experiment, percentages of larvae found on a leaf were compared to the percentage of leaf area of the respective leaf with  $\chi^2$  tests (PROC FREQ; SAS Institute, 1999), because differences in leaf size could affect the probability of random larval encounter with the leaves. If the proportions of larvae on the leaves were proportionate to respective leaf areas, then movement was considered random. Otherwise, larval movement was considered non-random, or directional. One-way ANOVA was utilized to analyze beet armyworm feeding damage on days 2, 4, and 7, and life-time damage. Feeding damage was also analyzed with repeated measures ANOVA. Beet armyworm adult female oviposition results were analyzed with one-way ANOVA. Data were not transformed unless otherwise noted. In all ANOVA analyses, if the null hypothesis was rejected at  $\alpha = 0.05$ , means were further separated by a two-tailed t-test with  $\alpha = 0.05$ .

## Results

### Nitrogen effect on cotton plant growth and nutritional quality

Plant growth was significantly affected by N supplied in the water (Table 2). Plants with higher N applied were taller, had greater dry shoot weight, dry root weight, and total biomass compared to those with low N input.

Treatment significantly affected percentage of total N in leaf blade dry mass, regardless of leaf position (L1/2:  $\chi^2 = 14.95$ ,  $P = 0.0019$ ; L4:  $\chi^2 = 16.71$ ,  $P < 0.001$ ; and L5:  $\chi^2 = 16.28$ ,  $P = 0.001$ ; Figure 1). Total N did not differ significantly between 196 and 280 p.p.m., regardless of leaf position (L1/2:  $\chi^2 = 0.01$ ,  $P = 0.9168$ ; L4:  $\chi^2 = 2.45$ ,  $P = 0.1172$ ; and L5:  $\chi^2 = 3.15$ ,  $P = 0.0758$ ; Figure 1). Plants receiving 196 p.p.m. N had consistently higher percentages of total N compared to those receiving 112 p.p.m. N, regardless of leaf position (L1/2:  $\chi^2 = 6.82$ ,  $P = 0.009$ ; L4:  $\chi^2 = 6.82$ ,  $P = 0.009$ ; and L5:  $\chi^2 = 4.81$ ,  $P = 0.028$ ; Figure 1). Likewise, the percentage of total N of leaf blade dry mass in 112 p.p.m. plants was higher than those receiving 42 p.p.m. N across the leaf positions (L1/2:  $\chi^2 = 5.77$ ,  $P = 0.0163$ ; L4:  $\chi^2 = 6.82$ ,  $P = 0.009$ ; and L5:  $\chi^2 = 6.82$ ,  $P = 0.009$ ; Figure 1).

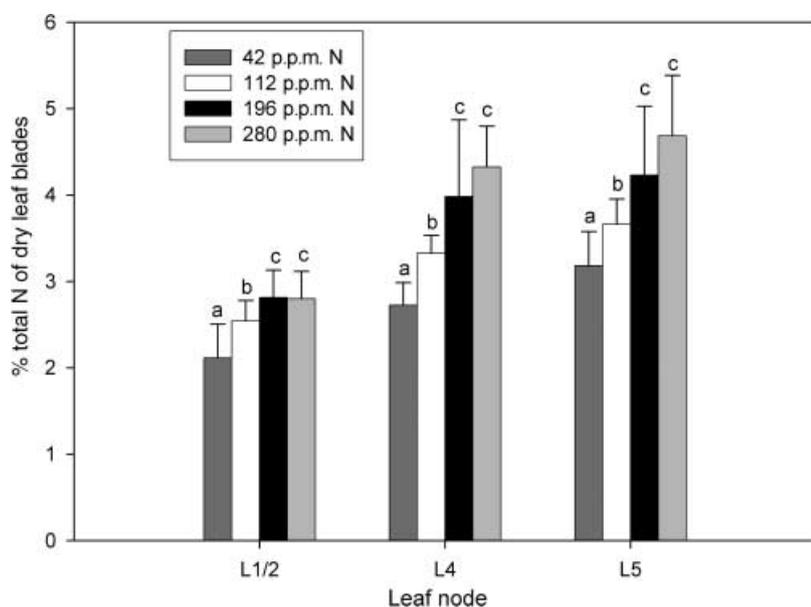
**Table 2** Cotton plant growth parameters in response to N availability in the greenhouse (cotton plants were at 3–5 mature leaf stages at time of experiment)

N treatment (p.p.m.)	Plant height mean $\pm$ SE (cm)	Dry shoot weight mean $\pm$ SE (g)	Dry root weight mean $\pm$ SE (g)	Total mass mean $\pm$ SE (g)								
42	29.67 $\pm$ 2.40a	2.01 $\pm$ 0.16a	0.51 $\pm$ 0.23a	2.53 $\pm$ 0.17a								
112	34.95 $\pm$ 2.26b	3.56 $\pm$ 0.42b	0.72 $\pm$ 0.08b	4.28 $\pm$ 0.49b								
196	40.13 $\pm$ 1.37c	5.15 $\pm$ 0.27c	0.89 $\pm$ 0.09bc	6.04 $\pm$ 0.33c								
280	43.08 $\pm$ 1.29c	6.46 $\pm$ 0.33d	0.87 $\pm$ 0.08c	7.34 $\pm$ 0.39d								
ANOVA												
Source	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P
Block	4	5.56	0.01	4	4.17	0.02	4	5.40	<0.01	4	5.62	<0.01
Treatment	3	18.2	<0.01	3	69.5	<0.01	3	12.1	<0.01	3	71.6	<0.01
Covariate <sup>1</sup>	1	13.2	<0.01									

<sup>1</sup>Plant height at the time of being first fertilized with corresponding nutrients. Means of plant height, dry shoot weight, dry root weight, and total mass were separated by pair-wise t-test after the null hypothesis of equality was rejected at  $\alpha = 0.05$ . Means within a column followed by different lower-case letters imply that they were significantly different at  $\alpha = 0.05$ .

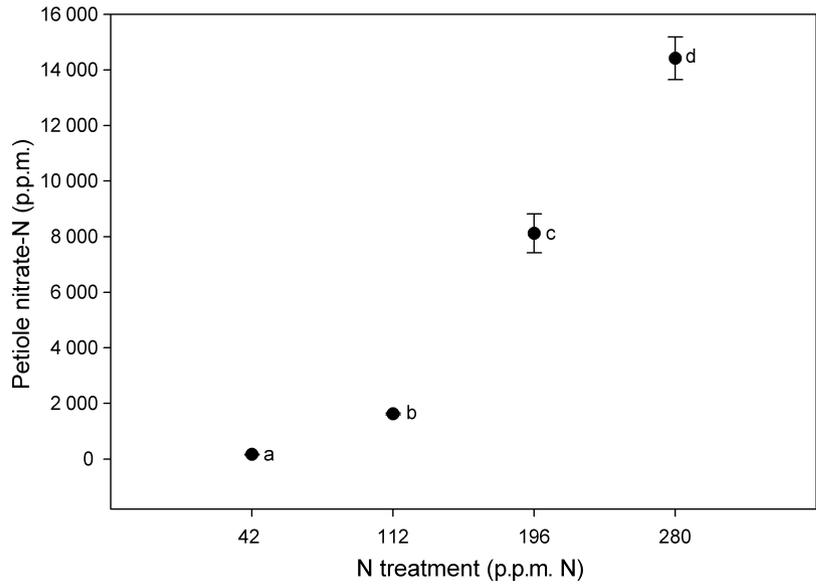
Leaf petiole nitrate-N status was strongly affected by treatment ( $F_{3,19} = 219.20$ ,  $P < 0.0001$ ; Figure 2). The average ( $\pm$  SE) nitrate-N amount in the 280 p.p.m. treatment was  $14\ 416.60 \pm 766.49$  p.p.m. The corresponding value for the 196 p.p.m. treatment was  $8\ 120.40 \pm 700.89$  p.p.m., which was significantly lower than that in 280 p.p.m. (Figure 2). Similarly, nitrate-N level was higher in plants of the 196 p.p.m. treatment compared to the 112 p.p.m. treatment ( $1622.00 \pm 33.20$  p.p.m.). The nitrate-N level of 112 p.p.m. was in turn higher than that of 42 p.p.m. ( $163.40 \pm 12.42$  p.p.m.; Figure 2).

*Hypothesis 1: beet armyworm development will be prolonged due to lower host plant N fertilization.* Nitrogen had a significant effect on beet armyworm development, regardless of the age of the larvae used at the onset of trials. In the neonate bioassay, larval weight differed across treatments only 3 days after beginning the trial (weight was not assessed on days 1 or 2) (Figure 3). The difference was greater on day 4. The effects of date, N treatment, and their interaction on larval weight were all significant ( $P < 0.0001$  in all cases; Figure 3). The interaction of date and N treatment was due to weight interactions of larvae in 196 and 280 p.p.m. N



**Figure 1** Percentage of total N of dried cotton leaf mass in relation to different N levels. L1/2: true leaves of node 1 and 2; L4: true leaf of node 4; and L5: true leaf of node 5. The bars represent averages ( $\pm$  SE) of five replicates. Different lower-case letters above N treatments of the same leaf position mean significant difference at  $\alpha = 0.05$ . Data were analyzed with a Kruskal–Wallis test.

**Figure 2** Nitrate-N (p.p.m.) of cotton petioles of different treatments. Different letters to the right of data points denote that the difference was significant at  $\alpha = 0.05$  level. Standard error bars of 42 and 112 p.p.m. N treatments do not appear, because they are smaller than symbols representing these two data points. Data were analyzed with analysis of variance (ANOVA) and means were separated with t-test after the overall null hypothesis was rejected at  $\alpha = 0.05$ .



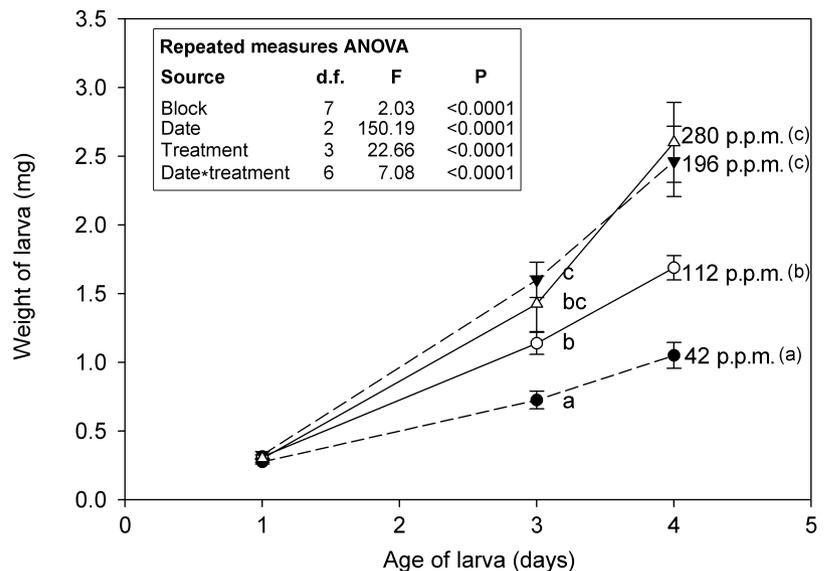
between days 3 and 4. On day 3, larvae in the 196 p.p.m. treatment weighed significantly more than those in 280 p.p.m., while on day 4 those in the 280 treatment weighed more.

In the cage trials that permitted intraplant movement of 3-day-old larvae, larvae reared on 196 and 280 p.p.m. N plants weighed significantly more than those reared on 112 and 42 p.p.m. N plants after 4 days of feeding (larvae were 7 days old) (Table 3). The same pattern was observed 3 days later (10-day-old larvae) (Table 3). The time from onset of bioassay to pupation and to adult emergence was significantly affected by N treatment ( $P < 0.0001$  in both

cases; Table 3). Pupal weight and percentage of larvae pupating were not significantly influenced by N (Table 3).

Nitrogen fertilization of host plants affected the number of instars that beet armyworm larvae underwent before pupation (Table 4). Of larvae reared on cotton plants in the 42 p.p.m. N treatment, an average ( $\pm$  SE) of  $96.0 \pm 2.45\%$  underwent six instars before pupation, which is significantly higher than the percentage ( $76.0 \pm 5.10\%$ ) of super-numerary larvae reared on 196 p.p.m. N treatment plants ( $F_{1,8} = 14.05, P < 0.0056$ ). Regardless of N treatment, however, more beet armyworm larvae reared on cotton plants

**Figure 3** Effects of cotton plant N application on short-term development of young *Spodoptera exigua* under no-choice conditions. Different letters to the right of data points of same day imply that the difference was significant at  $\alpha = 0.05$  level. Data were analyzed with analysis of variance (ANOVA) and means were separated with Duncan's test after the overall null hypothesis was rejected at  $\alpha = 0.05$ .



**Table 3** Growth and developmental times of *Spodoptera exigua* larvae on cotton plants grown with various N levels

N treatment (p.p.m. N)	Larval weight (mean ± SE) (mg)		Time to pupation <sup>1</sup> (mean ± SE) (days)	Time to adult emergence <sup>2</sup> (mean ± SE) (days)	Pupal weight (mean ± SE) (mg)	Percentage of larvae pupated (mean ± SE)							
	7-day-old	10-day-old											
42	9.00 ± 0.52a	42.34 ± 2.48a	15.82 ± 0.13a	23.04 ± 0.25a	107.59 ± 3.16	47.62 ± 7.43							
112	11.20 ± 0.29b	63.34 ± 1.60b	15.09 ± 0.18b	22.28 ± 0.20b	100.27 ± 3.74	53.57 ± 5.10							
196	13.10 ± 0.62c	82.19 ± 5.54c	14.72 ± 0.16bc	21.99 ± 0.30bc	101.28 ± 3.22	54.76 ± 7.01							
280	12.91 ± 0.44c	93.26 ± 8.65c	14.49 ± 0.10c	21.59 ± 0.19c	102.89 ± 1.93	63.10 ± 4.40							
ANOVA													
Source	d.f.	F	P	F	P	F	P	F	P	F	P	$\chi^2$	P
Block	6	0.64	0.70	1.06	0.42	0.91	0.51	2.12	0.07	2.80	0.0466	–	–
Treatment	3	14.25	<0.0001	17.68	<0.0001	16.21	<0.0001	10.06	<0.0001	0.88	0.4721	3.1899	0.3633

<sup>1</sup>Time from onset of bioassay to pupation.

<sup>2</sup>Time from onset of bioassay to adult emergence.

Means followed by different letters within a column imply that the difference was significant at  $\alpha = 0.05$  level. Percentage of larvae pupated was analyzed with Kruskal–Wallis test. Other data were analyzed with ANOVA and means were separated with Duncan's test after the overall null hypothesis was rejected at  $\alpha = 0.05$ .

underwent six instars (42 p.p.m. N treatment: fifth:sixth instar = 4%:96%,  $\chi^2 = 7.26$ , and  $P < 0.0071$ ; 196 p.p.m. N treatment: fifth:sixth instar = 24%:76%,  $\chi^2 = 6.90$ , and  $P < 0.0086$ ; Table 4) than is the case on artificial diets (Y Chen, unpubl.).

Developmental times of larvae were affected by the N levels of host plants on which they were reared (Table 4). The developmental time from egg hatch to the second instar, fifth instar, sixth instar, and pupation was significantly longer for larvae reared on 42 p.p.m. N cotton plants than corresponding times of larvae reared on 196 p.p.m. N host plants, although differences from egg hatch to the third and fourth instars were not significant among N treatments (to the second instar:  $F_{1,8} = 8.71$ ,  $P < 0.02$ ; to the third instar:  $F_{1,8} = 2.82$ ,  $P < 0.13$ ; to the fourth instar:  $F_{1,8} = 2.71$ ,  $P < 0.14$ ; to the fifth instar:  $F_{1,8} = 7.82$ ,  $P < 0.02$ ; to the sixth instar:  $F_{1,8} = 21.52$ ,  $P < 0.0017$ ; and to pupation:  $F_{1,8} = 41.88$ ,  $P < 0.0002$ ).

*Hypothesis 2: the life-time feeding damage of beet armyworm will be inversely related with host plant N fertilization rates.* Originally, there were 10 replicates for each treatment. Some replicates were excluded due to escape of larvae. So, only those replicates in which all three larvae were recovered (eight replicates for 42 and 280 p.p.m. N; seven for 112 and 196 p.p.m. N) at the end of trial were included.

Beet armyworm larvae consumed little leaf tissue before 7 days of age (Figure 4), when in the later portion of the third instar. The total area eaten by that time was less than 5 cm<sup>2</sup> per larva. The damage increased rapidly thereafter until pupation (Figure 4). The time from bioassay to pupation varied between 1 and 3 days among larvae. The life-time leaf areas consumed by each larva reared on 42, 112, 196, and 280 p.p.m. N plants were (mean ± SE) 64.7 ± 6.18, 60.1 ± 3.63, 54.6 ± 3.18, and 55.5 ± 2.61, respectively (Figure 4). The difference across treatments was not statistically significant ( $P = 0.11$ ). However, the difference

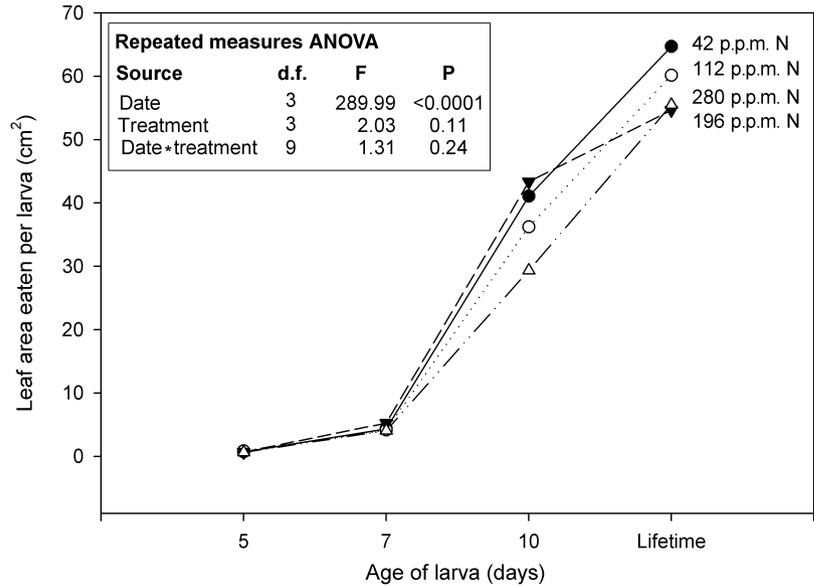
**Table 4** Supernumerary larval development of *Spodoptera exigua* due to low nutritional quality of cotton

N treatment	Number of larvae	Developmental time <sup>1</sup> (mean ± SE) (days)						Maximum larval instars <sup>2</sup>	
		Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Pupation	Fifth instar (%)	Sixth instar (%)
42 p.p.m. N	50	4.16 ± 0.18b	7.12 ± 0.19	9.44 ± 0.28	12.64 ± 0.43b	16.04 ± 0.41b	22.36 ± 0.29b	4.00 ± 2.45a	96.00 ± 2.45*
196 p.p.m. N	50	3.52 ± 0.12a	6.72 ± 0.15	8.90 ± 0.16	11.22 ± 0.27a	13.60 ± 0.32a	19.00 ± 0.43a	24.00 ± 5.10b	76.00 ± 5.10*

<sup>1</sup>Time from egg hatch to various developmental stages.

<sup>2</sup>*Spodoptera exigua* have 5–6 instars.

\*Within N treatment, significantly more *S. exigua* underwent a sixth instar before pupation. Data were analyzed with one-way analysis of variance (ANOVA). Percentages of individuals undergoing 5–6 instars before pupation were transformed ( $\arcsin \sqrt{x}$ ) before analysis.



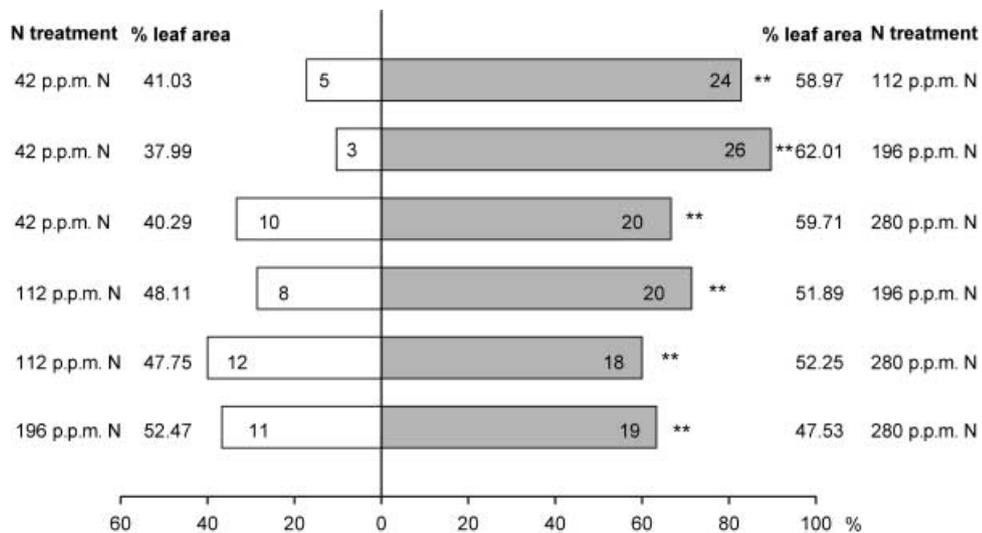
**Figure 4** Cumulative leaf damage by *Spodoptera exigua* larvae on cotton plants in relation to plant nitrogen. Data were analyzed with analysis of variance (ANOVA).

between highest damage (42 p.p.m. N) and lowest damage (196 and 280 p.p.m. N) reached some 10 cm<sup>2</sup> per larva, which was a ca. 15% difference.

*Hypothesis 3: beet armyworm larvae will prefer to feed on host plant tissues receiving higher N fertilization.* Nitrogen affected beet armyworm larval feeding choice (Figure 5). In all six combinations of two-choice tests between 42, 112, 196, and 280 p.p.m. N treatments, more larvae were found on the leaves with higher N content. Based on

the percentage of larvae found on a leaf compared to the percentage of leaf area, the movement of larvae was not random ( $P < 0.01$  in all cases) (Figure 5).

*Hypothesis 4: beet armyworm female will oviposit preferentially on host plants with higher N levels.* Nitrogen significantly affected beet armyworm female oviposition preference. There were significantly more egg masses laid on higher N cotton leaves (42 vs. 196 p.p.m. N:  $F_{1,14} = 21.00$ ,  $P < 0.001$ ; 112 vs. 196 p.p.m. N:  $F_{1,14} = 14.97$ ,  $P < 0.01$ ; Table 5). There



**Figure 5** Percentage of *Spodoptera exigua* larvae on cotton leaves with different N levels during two-choice tests. Numbers on the bar represent total count of larvae found on corresponding leaves of four replicates. \*\*Means  $P < 0.01$  in the two-choice test. Data were analyzed with a  $\chi^2$ -test.

**Table 5** The number (mean  $\pm$  SE) of egg masses and eggs laid on cotton test leaves by *Spodoptera exigua* female in oviposition choice tests<sup>1</sup>

	42 vs. 196 p.p.m. N		112 vs. 196 p.p.m. N	
	42 p.p.m. N	196 p.p.m. N	112 p.p.m. N	196 p.p.m. N
Number of egg masses	0.00 $\pm$ 0.00	1.50 $\pm$ 0.33**	0.38 $\pm$ 0.26	2.00 $\pm$ 0.33*
Number of eggs	0.00 $\pm$ 0.00	38.00 $\pm$ 7.78**	9.88 $\pm$ 6.50	57.75 $\pm$ 9.87*

<sup>1</sup>The third true leaves from cotton plants grown with two N treatments were used in the bioassay.

\*P<0.01; \*\*P<0.001.

were significantly more eggs laid on higher N cotton leaves (42 vs. 196 p.p.m. N:  $F_{1,14} = 23.84$ ,  $P < 0.001$ ; 42 vs. 196 p.p.m. N:  $F_{1,14} = 16.40$ ,  $P < 0.01$ ; Table 5). In a few cases, there were some egg masses laid on the test cages. But compared to the surface areas of the cage and the leaves, this was negligible.

## Discussion

As expected, greater N fertilization increased plant total N content in the study. Within a treatment, the percentages of total N in younger leaves were higher than those of older leaves. This may reflect the generally greater fitness value of younger leaves, and that nutrients of older leaves translocate nutrients to young leaves as leaves age (Ohnmeiss & Baldwin, 2000). Leaf petiole nitrate-N is a reliable indicator of cotton N status (Keisling, 1995; Weir et al., 1996), and N fertilization increased leaf petiole nitrate-N.

Increased N application enhanced plant growth and increased plant nutritional quality for *S. exigua* larvae. Larvae that had fed on higher N diets or host plants had greater growth rates, and shorter developmental times, which is similar to what has been found for other herbivores on other host plants (Mattson, 1980; Lindroth et al., 1995; Chen et al., 2004). *Spodoptera exigua* larvae were also capable of detecting the nutritional difference in various food plants and preferentially fed on host plants with higher nutritional quality. This is also similar to what has been found for other herbivores on other plants (White, 1984; Fox et al., 1990; Chen et al., 2004; Prudic et al., 2005).

The developmental times of *S. exigua* larvae feeding on lower N cotton plants were prolonged even though some of the larvae were fed on lower N plants for less than half of their larval developmental lives. The life-time feeding damage of *S. exigua* larvae on host plants with varying N levels were not statistically significant from each other, although larvae feeding on lower N plants tended to consume more plant tissue. The lack of compensatory feeding of *S. exigua* larvae on low nutritional quality foods was unexpected, because many lepidopteran herbivores have been shown to compensate for low nutritional quality

by increasing consumption amounts (Woods, 1999; Lavoie & Oberhauser, 2004; Lee et al., 2004a). Therefore, the reduced suitability of low N plants can increase feeding damage on these plants. Nevertheless, beet armyworm larvae are capable of choosing higher N plant tissues for feeding as shown in this study. Therefore, if beet armyworm larvae are able to choose and can move to higher N plants, overall feeding damage may be reduced on the less-preferred plants than on high N plants.

Furthermore, larvae on lower N plants would likely be exposed to higher mortality due to increased exposure to natural enemies through longer developmental times and increased movement. However, as found by DM Olson and AM Cortessero (unpubl.), cotton plants with too low or too high N and those that are water stressed and fed upon by the caterpillar *Helioverpa zea* (Boddie) (Lepidoptera: Noctuidae) are less attractive to the caterpillar's parasitoid, *Microplitis croceipes* Cresson (Hymenoptera: Braconidae). Although beet armyworm is not a host of *M. croceipes*, beet armyworm parasitoids may be similarly affected. Thus, N effects on the third trophic level may need to be considered before we more fully understand the relationship between N fertilization and cotton plant damage by beet armyworm.

Female moths in this study showed a significant oviposition preference for cotton plants with higher N applications. Female oviposition choice in large part determines the fate of the offspring, particularly in those insects with low-mobility immature stages. One would expect oviposition preference to coincide with larval suitability as shown in this study. However, this is not always the case (Courtney, 1981; Singer et al., 1994; Berdegué et al., 1998; Showler & Moran, 2003). The beet armyworm is a generalist herbivore with over 90 known host plant species (Pearson, 1982). Both positive and negative correlations between beet armyworm oviposition preference and offspring performance have been reported in the literature. In tests with different host plant species, Showler (2001) showed that beet armyworm adult females preferred pigweed, *Amaranthus hybridus* L. (Amaranthaceae), over cotton for oviposition, and that third-instar beet armyworm larvae also chose *A. hybridus* over cotton in choice tests. Nevertheless, in choices

between the host plant species *Chenopodium murale* L. (Chenopodiaceae) and *Apium graveolens* L. (Apiaceae), beet armyworm adult females preferred *C. murale* over *A. graveolens* for oviposition, although the development of larvae reared on *C. murale* was prolonged and pupal weights were lower in comparison to those reared on *A. graveolens* (Berdegué et al., 1998). Given choices between drought-stressed and control cotton, beet armyworm females oviposited more on drought stressed plants (Showler & Moran, 2003). This relationship was suggested earlier from field observations (Ruberson et al., 1994b; Ruberson, 1996). Again, larval performance expressed as larval survivorship was greater on non-stressed cotton plants that were less preferred for oviposition (Showler & Moran, 2003). There is so far no satisfactory explanation for the lack of a positive correlation between larval feeding performance and female oviposition preference in these studies. One possible explanation is intraspecific competition or interference in resource-limited areas, because in the preliminary experiment with beet armyworm females, they not only showed no oviposition preference between cotton plants with various N levels when six females were released per cage, but they also oviposited the majority of eggs on walls of cage and flower pots (Y Chen, pers. obs.). But this explanation cannot account for the lack of a positive correlation between larval feeding performance and female oviposition preference shown by Showler & Moran (2003). In that study, only individual females were used in choice tests. Other mechanisms such as selection for enemy-free space (Lawton & McNeill, 1979) or several possibilities, such as the parasite/grazer hypothesis proposed by Thompson (1988), may also account for the differential relationship between beet armyworm larval feeding performance and adult female oviposition choice on water-stressed and variable N levels in cotton. More work needs to be done to fully understand these phenomena.

The mechanisms by which N fertilization increases *S. exigua* larval growth and modifies larval feeding and female oviposition preference are unknown. It is possible that the variable N applications shifted the balance of the plants' protein to carbohydrate ratio (P:C), or reduced levels of plant defensive compounds, or a combination of the two. An appropriate P:C ratio is important for growth and development of many phytophagous insects (Simpson & Raubenheimer, 1993; Bede et al., 2007). *Spodoptera exigua* is a generalist herbivore and prefers higher protein diets than specialist herbivores, such as *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae) in choice tests (Lee et al., 2004b). The self-selected dietary P:C ratio for *S. exigua* larvae was 22:20 (Bede et al., 2007). *Spodoptera exigua* larvae have also been shown capable of distinguishing between glanded (containing constitutive defensive

terpenoid aldehydes) and glandless isogenic lines of cotton plants and preferentially chose to feed on glandless cotton plants (McAuslane & Alborn, 1998). Many plant-defensive allelochemicals have been reported to be decreased by N addition (Stout et al., 1998; Darrow & Bowers, 1999; Hemming & Lindroth, 1999; Schmelz et al., 2003; Prudic et al., 2005), so it is possible that some elements of preference are attributable to changes in plant defensive capacity. These qualitative and quantitative attributes may be malleable by varying N fertilization, and may further modify the herbivore-plant interactions.

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### References

- Bede JC, McNeil JN & Tobe SS (2007) The role of neuropeptides in caterpillar nutritional ecology. *Peptides* 28: 185–196.
- Benrey B & Denno RF (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. *Ecology* 78: 987–999.
- Berdegué M, Reitz SR & Trumble JT (1998) Host plant selection and development in *Spodoptera exigua*: do mother and offspring know best? *Entomologia Experimentalis et Applicata* 89: 57–64.
- Bianchi FJJA, Vlak JM, Rabbinge R & van der Werf W (2002) Biological control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouses: development of a comprehensive process-based model. *Biological Control* 23: 35–46.
- Buresh RJ, Austin ER & Craswell ET (1982) Analytical methods in N-15 research. *Fertility Research* 3: 37–62.
- Burton RL (1969) Mass rearing the corn earworm in the laboratory. USDA-ARS No. 33-134, USA, pp 1–8.
- Chen YZ, Lin L, Wang CW, Yeh CC & Hwang SY (2004) Response of two *Pieris* (Lepidoptera: Pieridae) species to fertilization of a host plant. *Zoological Studies* 43: 778–786.
- Chen YG, Ruberson JR, Lewis WJ & Bednarz C (2006) Herbivore feeding and induction of systemic resistance in cotton plants. *Proceedings of the 2006 Beltwide Cotton Conference (CD-ROM)*, pp. 1510–1520. National Cotton Council, Memphis, TN, USA.
- Clancy KM & Price PW (1987) Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. *Ecology* 68: 733–737.
- Courtney SP (1981) Coevolution of pierid butterflies and their cruciferous foodplants: III. *Anthocharis cardamines* (L.) survival, development and oviposition on different host plants. *Oecologia* 51: 91–96.
- Darrow K & Bowers MD (1999) Effects of herbivore damage and nutrient level on induction of iridoid glycosides in *Plantago lanceolata*. *Journal of Chemical Ecology* 25: 1427–1440.

- Dudt JF & Shure DJ (1994) The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology* 75: 86–98.
- Ehler LE (2007) Impact of native predators and parasites on *Spodoptera exigua*, an introduced pest of alfalfa hay in northern California. *BioControl* 52: 323–338.
- Eveleens KG, van den Bosch R & Ehler LE (1973) Secondary outbreak induction of beet armyworm by experimental insecticide applications in cotton in California. *Environmental Entomology* 2: 497–503.
- Feeny P (1976) Plant apparency and chemical defense. *Recent Advances in Phytochemistry* (ed. by LE Gilbert & PH Raven), pp. 1–40. Plenum Press, New York, NY, USA.
- Fox LR, Letourneau DK, Eisenbach J & Nouhuys SV (1990) Parasitism rates and sex ratios of a parasitoid wasp: effects of herbivore and plant quality. *Oecologia* 83: 414–419.
- Fye RE & McAda WC (1972) Laboratory studies on the development, longevity, and fecundity of six lepidopterous pests of cotton in Arizona. Technical Bulletin no. 1454. U.S. Department of Agriculture, Washington DC, USA.
- Glynn C, Herms DA, Egawa M, Hansen R & Mattson WJ (2003) Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. *Oikos* 101: 385–397.
- Hemming JDC & Lindroth RL (1999) Effects of light and nutrient availability on aspen: growth, phytochemistry, and insect performance. *Journal of Chemical Ecology* 25: 1687–1714.
- Hoagland DR & Arnon DI (1950) The Water Culture Method for Growing Plants Without Soil. Circular 347, California Agricultural Experiment Station. University of California, Berkeley, CA, USA.
- Jauset AM, Sarasúa MJ, Avilla J & Albajes R (1998) The impact of nitrogen fertilization of tomato on feeding site selection and oviposition by *Trialeurodes vaporariorum*. *Entomologia Experimentalis et Applicata* 86: 175–182.
- Jones JB, Jr (1997) *Hydroponics: A Practical Guide for the Soilless Grower*. St. Lucie Press, Boca Raton, FL, USA.
- Keisling TC (1995) Using cotton petiole nitrate-nitrogen concentration for prediction of cotton nitrogen nutritional status on a clayey soil. *Journal of Plant Nutrition* 18: 35–45.
- Kyllmar K, Carlsson C, Gustafson A, Ulén B & Johnsson H (2006) Nutrient discharge from small agricultural catchments in Sweden: characterization and trends. *Agriculture, Ecosystems and Environment* 115: 15–26.
- Lavoie B & Oberhauser KS (2004) Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. *Environmental Entomology* 33: 1062–1069.
- Lawton JH & McNeill S (1979) Between the devil and the deep blue sea: on the problem of being a herbivore. *A Symposium of the British Ecological Society* 20: 223–244.
- Lee KP, Raubenheimer D & Simpson SJ (2004a) The effects of nutritional imbalance on compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar. *Physiological Entomology* 29: 108–117.
- Lee KP, Simpson SJ & Raubenheimer D (2004b) A comparison of nutrient regulation between solitary and gregarious phases of the specialist caterpillar, *Spodoptera exempta* (Walker). *Journal of Insect Physiology* 50: 1171–1180.
- Lindroth RL, Arteel GE & Kinney KK (1995) Responses of three saturniid species to paper birch grown under enriched CO<sub>2</sub> atmospheres. *Functional Ecology* 9: 306–311.
- Mattson WJ Jr (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* 11: 119–161.
- McAuslane HJ & Alborn HT (1998) Systemic induction of allelochemicals in glanded and glandless isogenic cotton by *Spodoptera exigua* feeding. *Journal of Chemical Ecology* 24: 2861–2879.
- McGill D & Figueiredo (1993) Total nitrogen. *Soil Sampling and Methods of Analysis* (ed. by MR Carter), pp. 201–211. Lewis Publishing, Boca Raton, FL, USA.
- Mohaghegh J, DeClercq P & Tirry L (2001) Functional response of the predators *Podisus maculiventris* (Say) and *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) to the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae): effect of temperature. *Journal of Applied Entomology* 125: 131–134.
- Moran N & Hamilton WD (1980) Low nutritive quality as defense against herbivores. *Journal of Theoretical Biology* 86: 247–254.
- Ohnmeiss T & Baldwin IT (2000) Optimal defense theory predicts the ontogeny of an induced nicotine defense. *Ecology* 81: 1765–1783.
- Ojeda G, Tarrasón D, Ortiz O & Alcañiz JM (2006) Nitrogen losses in runoff waters from a loamy soil treated with sewage sludge. *Agriculture, Ecosystems and Environment* 117: 49–56.
- Pearson AC (1982) *Biology, Population Dynamics, and Pest Status of the Beet Armyworm (Spodoptera exigua) in the Imperial Valley of California*. PhD Dissertation, University of California, Riverside, CA, USA.
- Prudic KL, Oliver JC & Bowers MD (2005) Soil nutrient effects on oviposition preference, larval performance and chemical defense of a specialist insect herbivore. *Oecologia* 143: 578–587.
- Ruberson JR (1996) Environmental conditions and biological control of the beet armyworm. *Proceedings of the 1996 Beltwide Cotton Conference* (ed. by P Dugger & DA Richter), pp. 116–118. National Cotton Council, Memphis, TN, USA.
- Ruberson JR, Herzog GA, Lambert WR & Lewis WJ (1994a) Management of the beet armyworm (Lepidoptera: Noctuidae) in cotton: role of natural enemies. *Florida Entomologist* 77: 440–453.
- Ruberson JR, Herzog GA, Lambert WR & Lewis WJ (1994b) Management of the beet armyworm: integration of control approaches. *Proceedings of the 1994 Beltwide Cotton Conference* (ed. by P Dugger & DA Richter), pp. 857–858. National Cotton Council, Memphis, TN, USA.
- SAS Institute (1999) *SAS/STAT User's Guide*, 8th edn. SAS Institute, Cary, NC, USA.
- Schmelz EA, Alborn HA, Engelberth J & Tumlinson JH (2003) Nitrogen deficiency increases volicitin-induced volatile emission, jasmonic acid accumulation, and ethylene sensitivity in Maize. *Plant Physiology* 133: 295–306.
- Showler AT (2001) *Spodoptera exigua* oviposition and larval feeding preferences for pigweed, *Amaranthus hybridus*, over

- squaring cotton, *Gossypium hirsutum*, and a comparison of free amino acids in each host plant. *Journal of Chemical Ecology* 27: 2013–2028.
- Showler AT & Moran PJ (2003) Effects of drought stressed cotton, *Gossypium hirsutum* L., on beet armyworm, *Spodoptera exigua* (Hübner), oviposition, and larval feeding preferences and growth. *Journal of Chemical Ecology* 29: 1997–2011.
- Simpson SJ & Raubenheimer D (1993) The central role of the haemolymph in the regulation of nutrient intake in insects. *Physiological Entomology* 18: 395–403.
- Singer MC, Thomas CD, Billington HL & Parmesan C (1994) Correlates of speed of evolution of host preference in a set of twelve populations of the butterfly *Euphydryas editha*. *Ecoscience* 1: 107–114.
- Stiling P & Moon DC (2005) Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. *Oecologia* 142: 413–420.
- Stout MJ, Brovont RA & Duffey SS (1998) Effect of nitrogen availability on expression of constitutive and inducible chemical defenses in tomato, *Lycopersicon esculentum*. *Journal of Chemical Ecology* 24: 945–963.
- Thompson JN (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata* 47: 3–14.
- Udawatta RP, Motavalli PP, Garrett HE & Krstansky JJ (2006) Nitrogen losses in runoff from three adjacent agricultural watersheds with claypan soils. *Agriculture, Ecosystems and Environment* 117: 39–48.
- Weir BL, Kerby TA, Hake KD, Roberts BA & Zelinski LJ (1996) Cotton fertility. *Cotton Production Manual* (ed. by SJ Hake, TA Kerby & KD Hake), pp. 210–227. Division of Agricultural and Natural Resource, University of California, Oakland, CA, USA.
- White TCR (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63: 90–105.
- Wilkens RT, Spoerke JM & Stamp NE (1996) Differential responses of growth and two soluble phenolics of tomato to resource availability. *Ecology* 77: 247–258.
- Williams IS (1999) Slow-growth, high-mortality – a general hypothesis, or is it? *Ecological Entomology* 24: 490–495.
- Woods HA (1999) Patterns and mechanisms of growth of fifth-instar *Manduca sexta* caterpillars following exposure to low- or high-protein food during early instars. *Physiological and Biochemical Zoology* 72: 445–454.